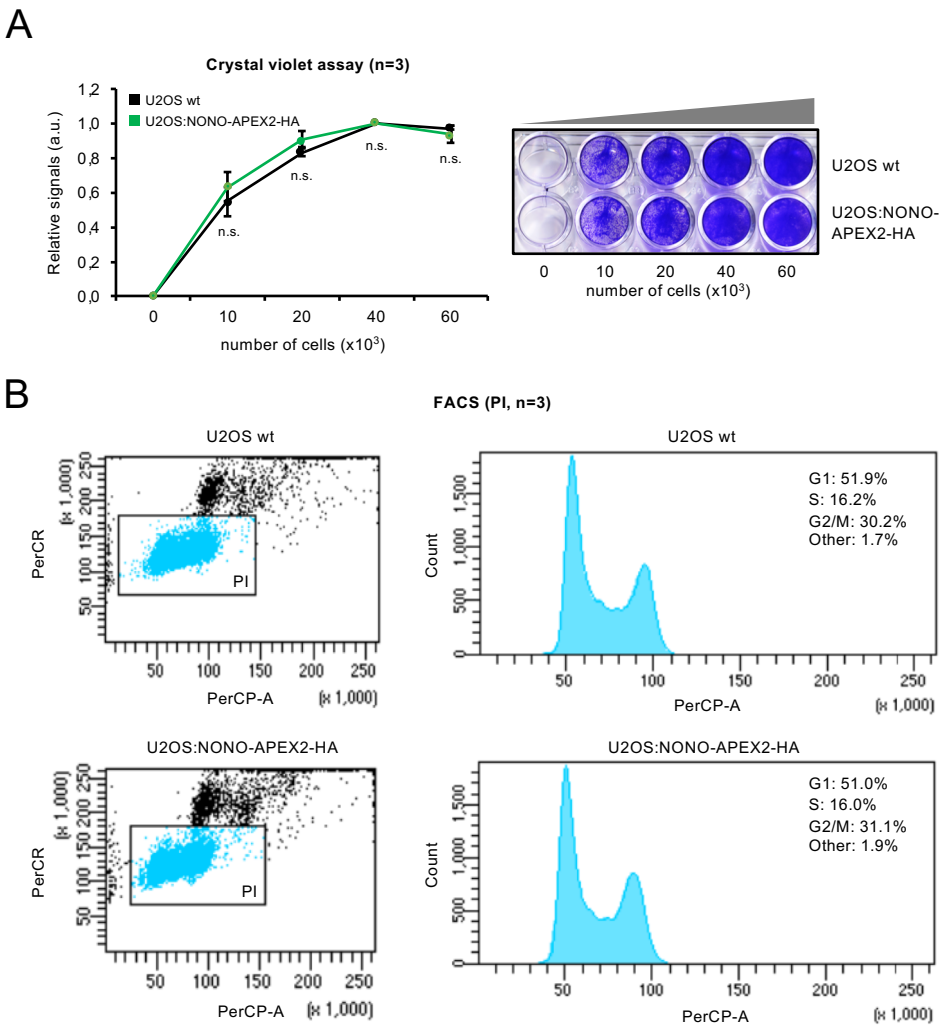
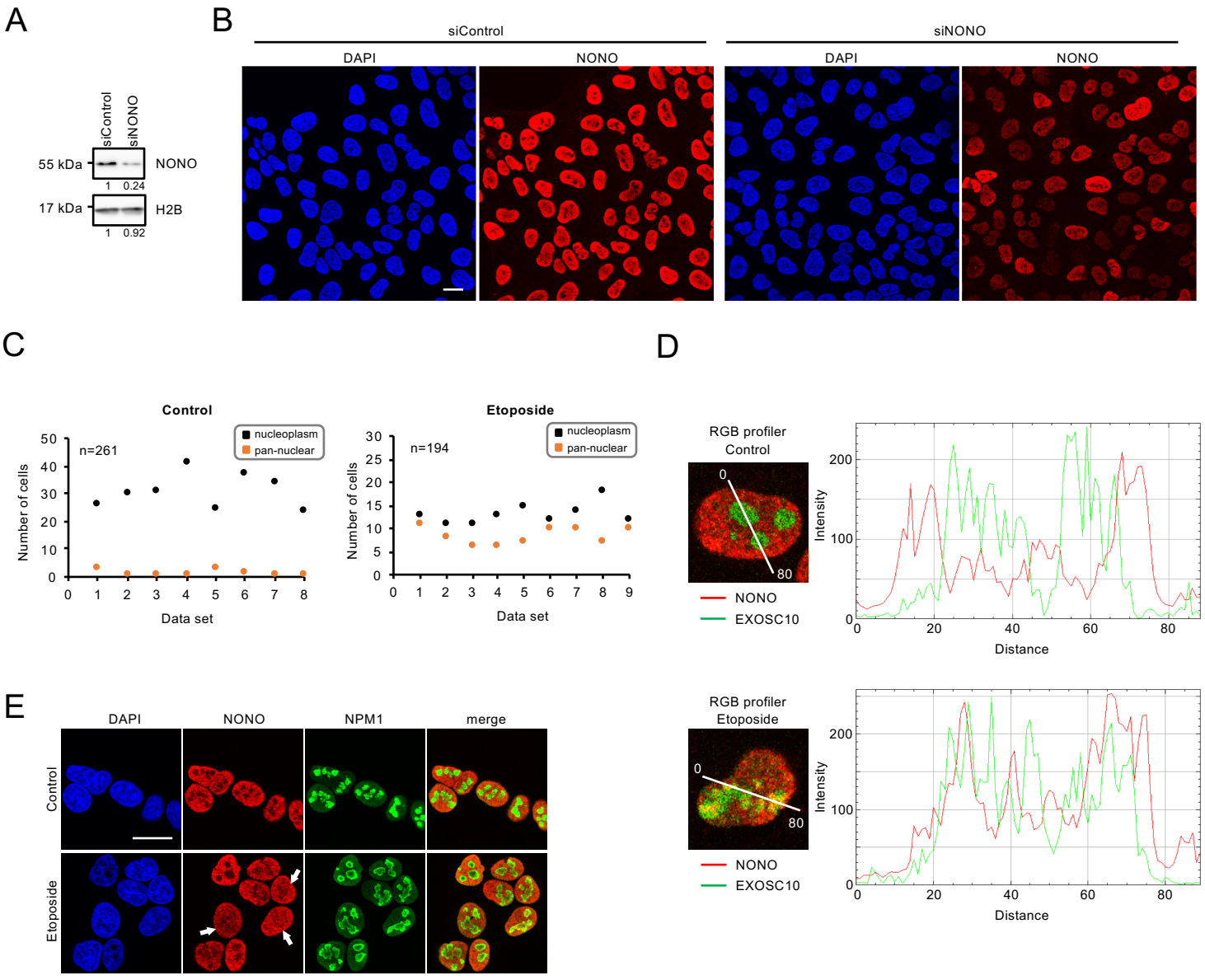
Supplementary Material

Supplementary Figure 1



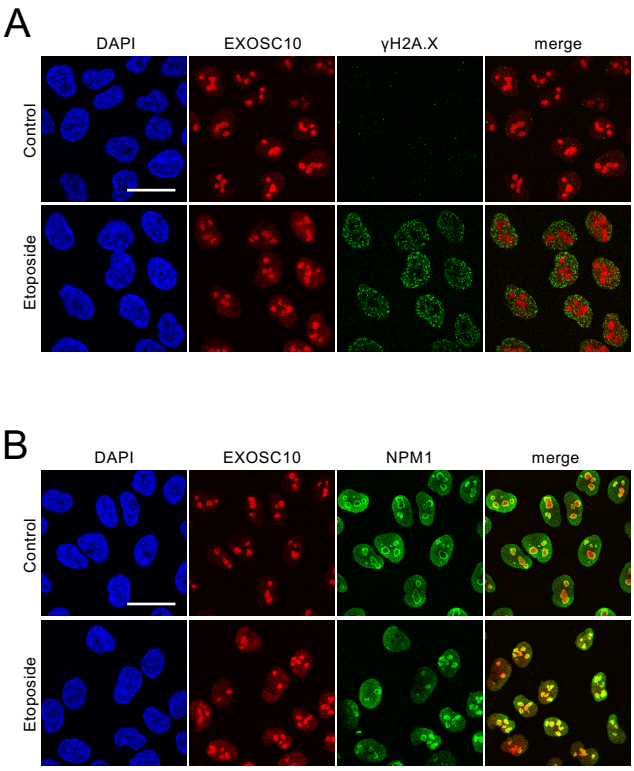
Supplementary Figure 1. **Proliferation and cell cycle analysis of U2OS:NONO-APEX2-HA cells.** (**A**) Crystal violet staining of wild type (wt) U2OS or U2OS:NONO-APEX2-HA cells are plotted as relative signals (left). Signal intensities were quantified by ImageJ and set to 1 for $40x10^3$ cells. a.u., arbitrary units; n.s., not significant. Significance was analysed by a Student's t-test. Scan of representative staining (right). (**B**) Cell cycle analysis by fluorescence-activated cell sorting (FACS). Gating of propidium iodide (PI)-stained wild type (wt) U2OS or U2OS:NONO-APEX2-HA cells for viable, non-duplet cells (left) and stratification for cell cycle phase (right). PerCP-A/ PerCR, forward/sideward scatter. A representative experiment is shown.

Supplementary Figure 2



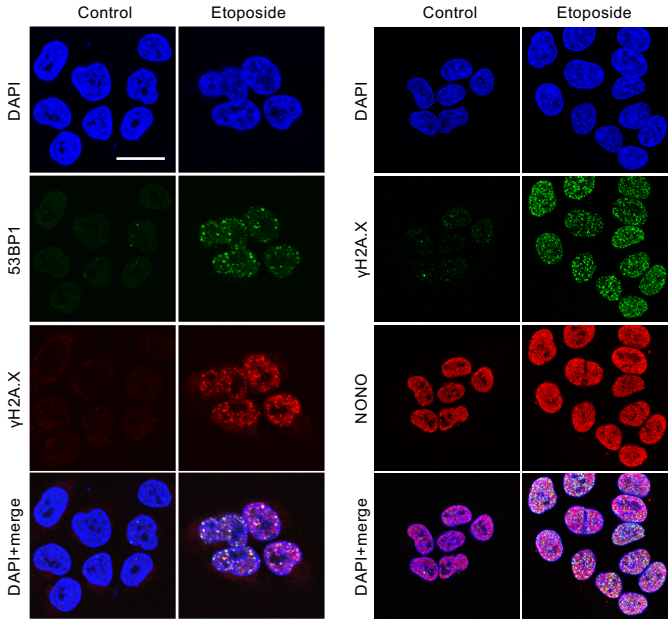
Supplementary Figure 2. **NONO antibody validation and assessment of subcellular NONO localisation in U2OS cells.** (**A**) Immunoblots detecting NONO upon transfection of small-interfering (si)RNA smart pools. H2B, histone 2B; loading control. Immunoblots were quantified by ImageJ. (**B**) Confocal imaging of NONO upon transfection of siRNA targeting NONO (siNONO) or non-targeting control (siControl). (**C**) Stratification of NONO subcellular localisation shows number of cells that display NONO staining in the nucleoplasm (black) or as pan-nuclear (orange). (**D**) RGB profiler plots for zoomed panels from Figure 2B. (**E**) Confocal imaging of NONO and Nucleophosmin 1 (NPM1) in the absence or the presence of Etoposide. Representative cells are shown. DAPI, 4‘,6-diamidino-2-phenylindol; scale bar, 10 µm; white arrowheads, pan-nuclear NONO staining.

Supplementary Figure 3



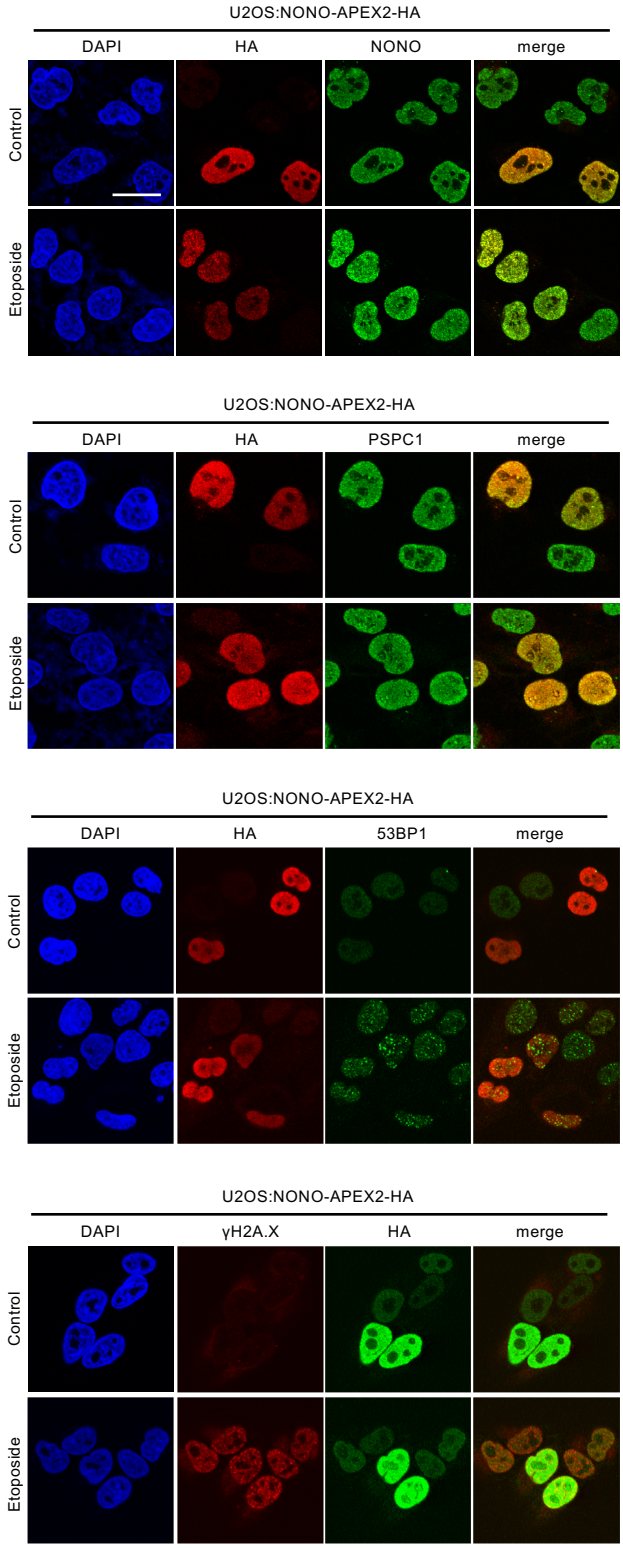
Supplementary Figure 3. **Preservation of nucleolar integrity upon short-term treatment with Etoposide in U2OS cells.** (**A**) Confocal imaging of EXOSC10 and γH2A.X in the absence or the presence of Etoposide. (**B**) Confocal imaging of EXOSC10 and NPM1 in the absence or the presence of Etoposide. Representative cells are shown. DAPI, 4‘,6-diamidino-2-phenylindol; scale bar, 10 µm.

Supplementary Figure 4



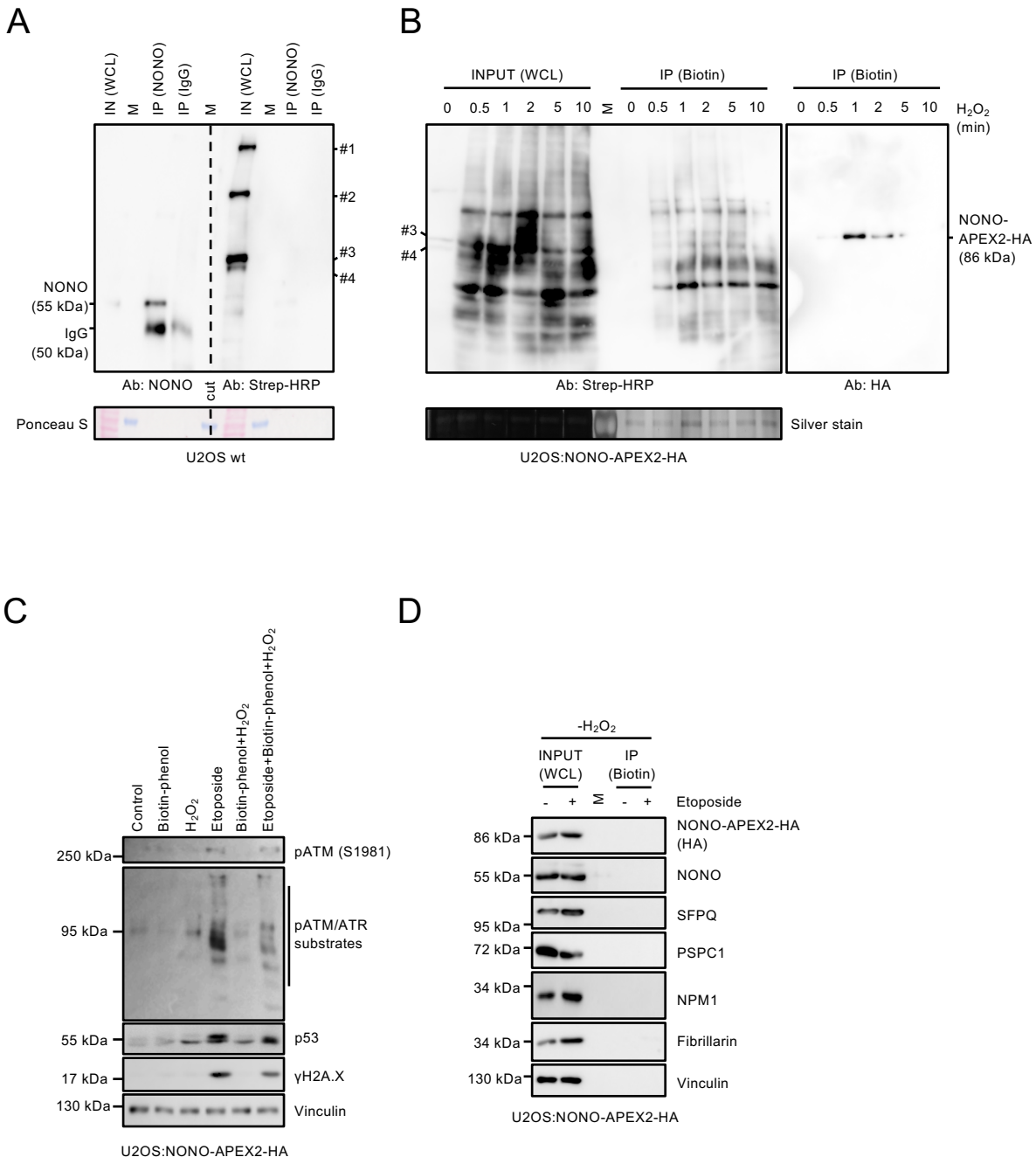
Supplementary Figure 4. **Colocalisation of γH2A.X with 53BP1, but not NONO in U2OS cells.** Confocal imaging of the p53-binding protein 1 (53BP1) and γH2A.X (left) or γH2A.X and NONO (right) in the absence or the presence of Etoposide. Representative cells are shown. DAPI, 4‘,6-diamidino-2-phenylindol; 4OHT, 4-hydroxy-tamoxifen; scale bar, 10 µm.

Supplementary Figure 5



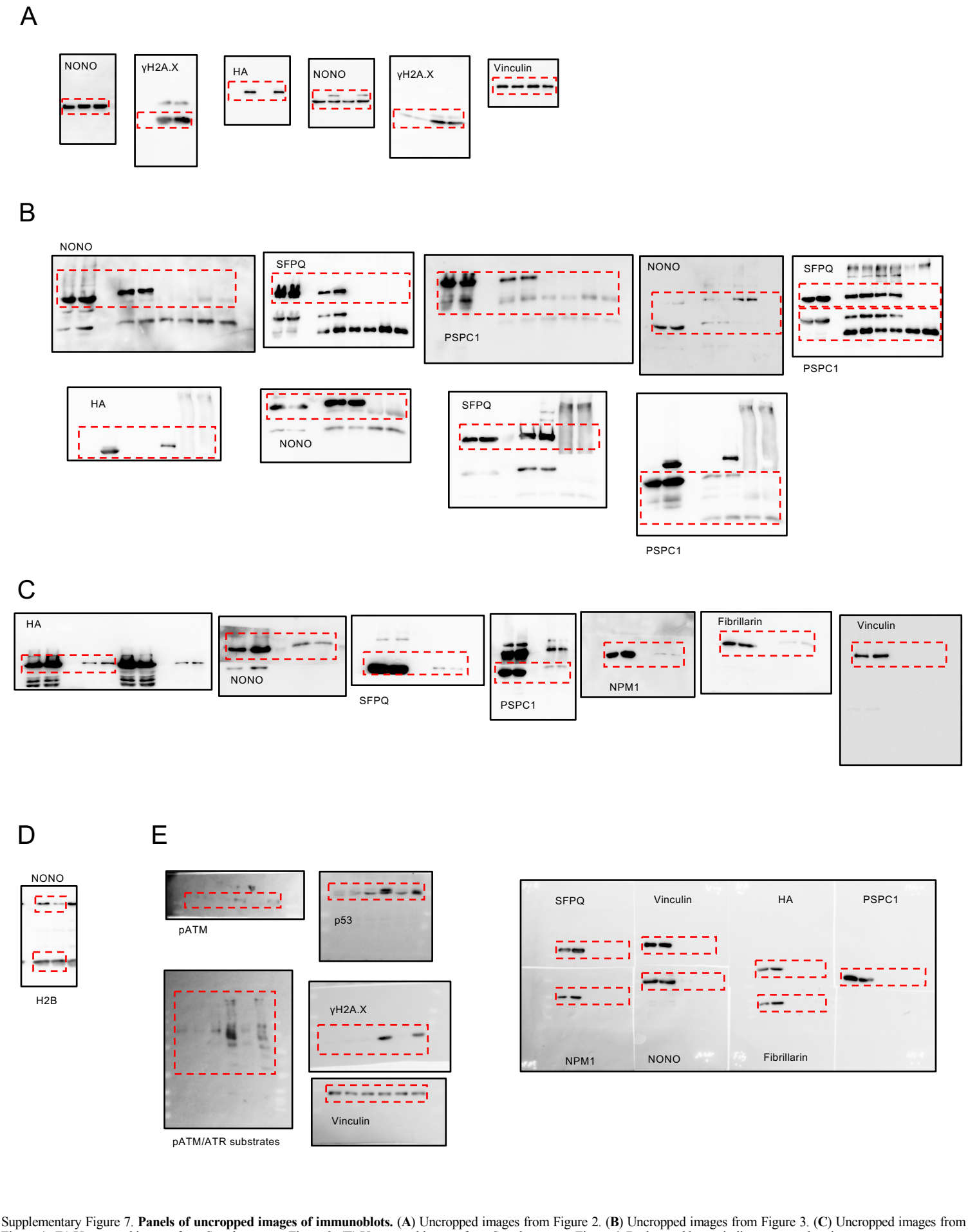
Supplementary Figure 5. **Colocalisation of NONO-APEX2-HA with NONO and PSPC1, but not 53BP1 and γH2A.X in U2OS:NONO-APEX2-HA cells.** Confocal imaging of NONO-APEX2-HA with NONO, PSPC1, 53BP1 or γH2A.X (top to bottom) in the absence or the presence of Etoposide. Representative cells are shown. DAPI, 4‘,6-diamidino-2-phenylindol; scale bar, 10 µm.

Supplementary Figure 6



Supplementary Figure 6. **Quality control for *in vivo* proximity labeling.** (**A**) Immunoblot detecting NONO or biotinylated proteins (Strep-HRP) from whole cell lysates (WCL) or upon immunoprecipitation (IP) from U2OS wild type (wt) cells. #1, CoA carboxylase (280 kDa); #2, Pyruvate carboxylase (128 kDa); #3, Propionyl-CoA carboxylase (74 kDa); #4, β-methylcrotonyl-CoA carboxylase (72 kDa); Ab, antibody; Strep-HRP, streptavidin-horse radish peroxidase; M, protein standard; Ponceau S, loading control; IgG; immunoglobulin; dashed line, cut membrane. (**B**) Immunoblots detecting biotinylated proteins (Strep-HRP) or NONO-APEX2-HA from WCL or upon IP from U2OS:NONO-APEX2-HA cells treated in a hydrogen peroxide (H_2O_2) time kinetic. Silver stain, loading control. (**C**) Immunoblots detecting Ser1981-phosphorylated ataxia telangiectasia mutated (pATM), substrates of ATM/ataxia telangiectasia mutated-related (pATM/ATR substrates), p53 and γH2A.X from WCL after incubation with Biotin-phenol (0.5 mM, 30 min), H_2O_2 (1 mM, 1 min), Etoposide (20 μM, 2 h) or combinations thereof. Vinculin, loading control. (**D**) Immunoblots detecting indicated proteins from WCL or after IP with or without Etoposide (20 μM, 2 h) in the absence of H_2O_2.

Supplementary Figure 7



Supplementary Figure 7. **Panels of uncropped images of immunoblots.** (**A**) Uncropped images from Figure 2. (**B**) Uncropped images from Figure 3. (**C**) Uncropped images from Figure 4. (**D**) Uncropped images from Supplementary Figure 2. (**E**) Uncropped images from Supplementary Figure 6. Broken red boxes indicate cropped regions.

Supplementary Table 1

Primer	Sequence (5'-3')
APEX2 Entry-fwd	ccTTAATTAAcgACGCGTGGTGGTGGTTCTggaaagtcttac
APEX2 Entry-rev	acactagtctaGGCGTAATCTGGAACATCGTATGGGTAggcatcagcaaa
NONO PCR-fwd	ccTTAATTAAccaccatgcagagtaataaaacttttaacttggagaagca
NONO PCR-rev	cgcgACGCGTgtatcggcgacgtttgtttggg
NEAT1 RT-qPCR-fwd	TTGACCAACGCTTTATTTTC
NEAT1 RT-qPCR-rev	TTACCAACAATACCGACTCC

Supplementary Table 1. **Primers for PCR cloning and RT-qPCR used in this study.** The APEX2 Entry-fwd primer contains a linker (GGTGGTGGTTCT), the APEX2 Entry-rev primer contains a stop codon and an HA tag (ctaGGCGTAATCTGGAACATCGTATGGGTA). The NONO PCR fwd primer contains a Kozak sequence and a start codon (ccaccatg). The NEAT1 primers target both short and long NEAT1 isoforms.

Supplementary Table 2

Antibody	Source
anti-NONO	Proteintech, 11058-1-AP
anti-EXOSC10	Abcam, ab50558
anti-γH2A.X (S139)	Merck Millipore, 05-636
anti-γH2A.X (S139)	Cell Signaling Tech., 2577
anti-NPM1	Abcam, ab10530
anti-HA tag	Abcam, ab9110
anti-HA tag	Biolegend, 901501
anti-Fibrillarin	Abcam, ab911
anti-Vinculin	Sigma, V9131
anti-SFPQ	Abcam, ab177149
anti-PSPC1	Proteintech, 16714-1-AP
anti-H2B	Abcam, ab1790
anti-53BP1	Novus, NB100-304
anti-p53	Santa Cruz, sc-126, DO-1
anti-phospho-ATM (S1981)	Abcam, ab81292
anti-phospho-ATM/ATR Substrate (S*Q)	Cell Signaling Tech., 9607
anti-IgG control	Proteintech, 30000-0-AP
anti-Streptavidin-HRP	Cytivia, RPN123
anti-rabbit-IgG-HRP	Cytivia, NA934
anti-mouse-IgG-HRP	Cytivia, NA931
Alexa fluor 568-goat-anti-rabbit-IgG	Invitrogen, A11036
Alexa fluor 488-goat-anti-mouse-IgG	Invitrogen, A11001
Alexa fluor 546-donkey-anti-mouse-IgG	Invitrogen, A10036
Alexa fluor 488-donkey-anti-mouse-IgG	Invitrogen; A21206
Avidin-neutravidin-tetramethylrhodamine (NeutrAvidin-568)	Invitrogen, A6373

Supplementary Table 2. **Primary and secondary antibodies used in this study.**